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Pulmonary Function and Pathology in Cats Exposed 28 Days to Diesel Exhaust

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16. ABSTRACT

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PULMONARY FUNCTION AND PATHOLOGY IN CATS EXPOSED 28 DAYS TO DIESEL EXHAUST

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Young adult male cats were exposed 28 days, 20 hrs per day, to a 1:14 dilution of diesel exhaust emissions. Following termination of exposure, the following pulmonary function measurements were carried out: lung volumes, maximum expiratory flow rates (MEF), MEF at 50%, 25% and 10% of vital capacity (VC): forced expiratory volume (FEV) after 0.2, 0.3 and 0.4 sec, dynamic compliance, resistance and helium washout at 25, 50, 75, and 100 breaths per min. The only significant functional change was a decrease in MEF at 10% of VC ($P \times .02$). The lungs of the exposed cats appeared charcoal grey with frequent focal black spots visible on the pleural surface. Pathologic changes in the exposed cats included a predominantly peribronchiolar localization of black-pigmented macrophages within the alveoli producing a focal pneumonitis or alveolitis. In general, evidence of serious lung damage was not observed following the 28-day exposure period.

INTRODUCTION

The use of diesel engines has increased greatly in recent years. Diesel engines provide the power for most large trucks, trains, heavy earth moving, construction and farm machinery. Diesel engines have largely supplanted gasoline engines in underground mines with the exception of coal mines. More recently, the number of diesel powered automobiles has also shown a significant increase. Due to the greater efficiency of fuel utilization of diesel powered vehicles, this trend will probably continue.

Although many of the pollutants emitted by diesel engines have been studied, the toxicological and carcinogenic effects of whole exhaust cannot be accurately predicted. For example, many of the pollutants are adsorbed, in part, on the particulate matter (Stokinger, 1973). The association of the pollutants with the particulate can alter the depth of penetration and residenct time of

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the pollutants in the lungs (Task group, 1966; Creasia *et al.*, 1976). Diesel exhaust also contains a wide variety of both aliphatic and aromatic compounds (Karasek *et al.*, 1974), the concentration of which and even the chemical structure are not well defined. Finally, the effects of a combination of pullutants cannot be predicted with certainty from studies of individual pollutants.

The present study was designed to evaluate, in cats, the effects of 28 days exposure to a 1:14 dilution of diesel exhaust upon pulmonary function and pathology.

METHODS

Male weanling cats, born and maintained in a desease free environment and inbred for several generations, were purchased from Liberty Labs, Liberty Corners, NJ. The cats were uniform in size and varied in age by less than two weeks. The cats were exposed in stainless steel chambers 1.38 meters square with an interior volume of 2.83 cu. m. Details of the chamber construction have been published previously (Himmers *et al.*, 1968). Each chamber contained a wire mesh floor and a half shelf of similar material covering one-half the inside area one meter above the floor. The cats were allowed to roam free in the chambers. Purina Cat Chow and water were provided *ad libitum* along with a litter box. Four chambers were used, housing 8 to 9 animals each. Two contained diesel exhaust and two CBR filtered and conditioned air. Care of the animals and cleaning of the chambers were carried out between 4:30 and 8:30 P.M., while the engines were not operating. Diesel exhaust was produced with a six cylinder 3.2 liter engine manufactured by Nissan Motor Company. The engine was mounted on a dynamometer base plate and coupled to a Chrysler torque-flite automatic transmission, which in turn was connected to a dynamometer power adsorption unit. The engine was cycled continuously at speeds corresponding to a modified "California" Cycle (Federal Register, 1966). A single lot of No. 2 diesel fuel was purchased for the study. All exhaust passed through a muffler before being diluted with 14 parts CBR filtered and conditioned air. After passing through the dilution tube, the diluted exhaust entered a 19.3 cu. m. mixing chamber from which it was piped to the exhaust chambers. The normal rate of air circulation through the chamber was 15 volumes per hr or 0.7/cu m/min. The temperature in the chamber was maintained at 24°C and the relative humidity near 50 percent.

An on-line monitoring system was used to measure CO, CO_2,NO, NO_2 and SO_x. Each gas was measured hourly in each chamber while the engines were operating for a maximum of 20 measurements per chamber per day. Total suspended particulates and sulfate were measured daily on week days for each chamber. Total hydrocarbons (THC) were measured daily from a single chamber.

Eighteen animals were exposed to raw diesel exhaust and 17 to clean air for 28 consecutive days, for 20 hrs per day. They were staged into the chambers at the rate of 4 per day. Following completion of exposure, each cat was removed from the chamber and anesthetized with about 35 mg/kg sodium pentobarbital iv. Additional amounts were given as needed to maintain an

adequate level of anesthesia. To prevent excess salivation, 0.5 mg atropine sulfate in one cc was injected im following induction of anesthesia. A tracheostomy was performed by making a cross sectional incision in the trachea just distal to the larynx. A thin-walled teflon catheter matching the size of the trachea was ligated in place. A saline filled catheter was implanted into the pleural cavity. The catheter was inserted by puncturing the body wall through the intercostal space between the fourth and fifth rib with a 10 guage needle. The catheter was inserted through the needle and sutured to the body wall after removal of the needle.

For measurement of expiratory flow-volume curves, dynamic compliance, resistance and pulmonary diffusing capacity, the cats were placed in a specially constructed small animal plethysmograph with the tracheal cannula ducted to the exterior. Transpulmonary pressure was measured with a Statham PR-23-20-300 pressure transducer connected to the intrapleural catheter. Airflow was measured with a Fleisch No. 0 pneumotochometer connected to a Statham PM 5 pressure transducer. Volume was obtained by integration of the flow signal. Data was recorded with an Electronics for Medicine Model DR-8 recorder.

To measure dynamic compliance and resistance, the door was sealed and the pressure in the plethysmograph cycled from -10 to +10 cm H_20 at a rate of 40 breaths per min. Compliance and resistance were calculated according to the method of Amdur and Mead (1956). Expiratory flow-volume measurements were then recorded during a rapidly induced pressure change for -60 to $^{+}$60 cm H_20. At least 4 maximal expirations were recorded, two as flow-volume loops and two as flow and volume versus time.

Pulmonary diffusing capacity was measured using the single breath method. The cat was forced to expire to residual volume by increasing plethysmograph pressure to 60 cm H_2O. A gas mixture containing 10% He and 0.311% $C^{18}0$ was then inspired to vital capacity by decreasing plthysmograph pressure to -60 cm H_2O. After 10 sec of breath-holding, the cat was again forced to expire to residual volume. Helium and $C^{18}0$ composition of the expired air were monitored continuously with a Perkin Elmer Model MGA-1100 Medical Gas Mass Spectrometer. Pulmonary diffusing capacity and residual volume were calculated using the method of Ogilvie *et al.* (1957). The calculations were based upon He and $C^{18}0$ concentrations at one-third of VC.

After completion of diffusion measurement, the cat was connected to a Harvard Model 661 animal respirator and ventilated with the stroke volume set at 15% of VC. A gas mixture containing 10% He in air was inspired until an asymptote in expired He concentration was reached. The respirator was then switched to room air and the number of breaths required to reduce He to 1% of peak concentration recorded. The procedure was repeated at 25, 50, 75 and 100 breaths per min.

For pathological evaluation of the lungs, gross lesions, if any, were recorded. The tissues were then trimmed, imbedded with paraplast, cut at 4-6 um and stained with hematoxylin eosin. Approximately 3 to 11 tissues on 2 to 5 slides were evaluate from each animal. The tissurs sectioned included the left and right diaphragmatic, intermediate, left apical, right cardiac lobes, upper trachea, the trachea at its bifurcation and the bronchial lymph nodes.

RESULTS

Analysis of chamber pollutant concentrations are shown in Table 1. The first four gases were measured hourly in both exhaust chambers. Since concentrations were nearly identical in both chambers, the means from only on of the chambers are shown. Since every chamber was not sampled every day for SO_2, sulfate and particulate, the measurements taken from all ten raw exhaust chambers were averaged. These included the two chambers used for cat exposures as well as eight other chambers. Total suspended particulates were measured daily on weekdays from one chamber. Ozone and ammonia concentrations were monitored periodically but concentrations were very low with ozone usually below the detection limits.

TABLE 1. Analysis of Major Chamber Contaminants

Parameter	Units	Mean	± SEM	N
CO_2	Percent	0.261	0.008	575
CO	ppm	14.61	0.90	575
NO	ppm	6.13	0.18	688
NO_2	ppm	2.13	0.09	378
SO_2	ppm	2.10	0.21	149
THC	ppm	31.56	1.25	36
Sulfate	mg/m^3	0.577	0.019	236
Particulate	mg/m^3	6.40	0.36	230

The mean weight for clean air cats was 3.29 kg at the start of exposure and increased to 3.60 kg after 4 weeks exposure. Exhaust cats avaraged 3.22 kg initially with their mean weight increasing to 3.50 kg at the end of exposure. A significant treatment x time interaction could not be detected using a two way analysis of variance, indicating that the rate of gain in body weight did not differ significantly between the two groups.

Hematological measurements are shown in Table 2. Significant differences in hemoglobin, hematocrit, red cell counts or white cell counts could not be detected between exposed and control cats. Individual leucocyte counts (not shown) varied considerably and were not analyzed statistically.

Lung volumes and pulmonary function parameters are shown in Table 3. TLC and VC were nearly identical in the exhaust and control cats. As a result, it was considered unnecessary to adjust the various pulmonary function para meters for lung volume. RV was somewhat greater in the exhaust exposed cats but not significantly so ($P=0.19$).

MEF was not significantly influenced by previous exhaust exposure. Maximum flows at 50%, 25% and 10% of VC were slightly lower in the exhaust exposed cats with differences becoming significant, $P<0.02$ at 10% of VC.

FEV/VC after 0.2 and 0.3 seconds showed very little difference between groups. The fraction of VC expired after 0.4 sec was slightly less in exhaust exposed cats but not significantly so ($P<0.25$).

Exposure to diesel exhaust failed to result in significant changes in either dynamic compliance, resistance, pulmonary diffusing capacity or the number of breaths required for helium washout. Neither group of cats showed a frequency dependence of helium washout up to 75 breaths per min. At 100 breaths per min, a small increase occurred in both groups in the number of breaths necessary to reduce expired helium to 1% of peak concentration.

TABLE 2. Hematology Measurements in Cats Following 28 Days Exposure to Diesel Exhaust

Parameter	Control ±	N	Exhaust ± Sd	N	P
Hb	11.0 ± 1.32	16	11.0 ± 1.38	15	.90
HCT	33.1 ± 3.97	16	31.5 ± 3.98	15	.28
RBC*	7.268 ± 0.972	16	7.629 ± 0.857	15	.28
WBC/mm³	15943 ± 11854	16	14620 ± 4590	15	.69

*$\times 10^6$/mm³

TABLE 3. Pulmonary Function Parameters in Cats Following 28 Days Exposure to Diesel Exhaust

Measurement[1]	Control ± SD	N	Exhaust ± SD	N	P
TLC (cc)	254 ± 65	16	257 ± 47	16	0.88
VC (cc)	214 ± 61	16	214 ± 38	16	1.00
RV (cc)	39.9 ± 8.1	16	45.0 ± 12.1	16	0.19
MEF[2] (LPM)	53.8 ± 10.9	16	54.7 ± 8.7	16	0.79
MEF_{50} (LPM)	44.7 ± 11.8	16	40.5 ± 11.9	16	0.33
MEF_{25} (LPM)	29.5 ± 9.1	16	25.1 ± 8.2	16	0.16
MEF_{10} (LPM)	15.6 ± 5.2	16	11.4 ± 4.6	16	0.02
FEV[3].2/VC%	45.1 ± 9.3	16	45.2 ± 8.9	16	0.96
FEV .3/VC%	76.0±9.9	16	75,9 ± 10.2	16	1.00
FEV .4/VC%	92.5 ± 4.9	16	90.0 ± 7.1	16	0.25
DLCO (cc/min/mm Hg)	1.47 ± 0.14	15	1.38 ± 0.20	13	0.51
Cdyn[4] (cc/cm H_2O)	3.60 ± 1.90	16	3.37 ± 1.33	16	0.69
RL[5] (cm H_2O/L/min)	0.404 ± 0.207	16	0.387 ± 0.194	16	0.81
He W_{25}[6]	10.73 ±2.28	15	10.53 ± 1.41	15	0.77
HeW_{50}	10.80 ±2.30	15	10.80 ±1.78	15	1.00
HeW_{75}	11.73 ± 2.58	15	11.73 ± 2.19	15	1.00
HeW_{100}	13.47 ±2.70	15	14.00 ±3.38	15	0.64

[1]All measurements corrected to STPD when applicable
[2]MEF Maximum expiratory flow at fractions of vital capacity listed.
[3]FEV Forced expiratory volume at fractions of seconds listed.
[4]Cdyn Dynamic compliance.
[5]RL Resistance.
[6]HeW Helium washout. Number of breaths required to reduce He to 1% of peak concentration at breathing rates/min. listed.

A necropsy, the lungs of the cats exposed to diesel exhaust appeared charcoal grey with some lungs containing 0.5 - 1.0 mm black foci randomly distributed over the pleural surface. Histopathological examination of the respiratory tract by light microscopy revealed the presence of black pigmented alveolar macrophages free within the lumen of the alveoli or adherent to the mucosa of the trachea, bronchi or bronchioles. Rare granules of black pigment were noted in the tracheal or bronchiolar epithelium. The predominant finding in the cats exposed to diesel exhaust was the accumulation of clusters of 1-50 black pigmented alveolar macrophages throughout the lung, but particularly in peribronchiolar locations (Fig. 1). Occasional polymorphonuclear leucocytes were present with the macrophages. In the affected areas, focal hypertrophy of the alveolar lining cells and interstitial thickening were noted. In most of the exposed cats, the subscapular and medullary sinuses of the tracheobronchial lymph nodes contained aggregates of black pigment.

FIGURE 1. Lung section showing accumulation of cluster of black pigmented alveolar macrophages, focal hypertrophy of adjacent alveolar lining cells and interstitial thickening.

DISCUSSION AND CONCLUSIONS

The only significant decrement in pulmonary function detected in the exhaust exposed cats was a decrease in MEF at 10% of VC. Although not differing significantly from controls, MEF's at 50% and 25% of VC as well as FEV .4/VC, also showed a trend toward lower values. The primary determinants of maximum expiratory flow rates near the end of expiration are static recoil pressure and small airway caliber (Bouhuys, 1974). For example, MEF at 50% of VC or less was decreased under conditions resulting in either small airway obstruction or constriction such as smoking (DaSilva and Hamosh, 1973; McFadden *et al.*, 1974) and chronic exposure to coal dust (Moorman *et al.*, 1975). MEF at low lung volumes was also decreased in subclinical emphysema in which loss of elastic recoil is one of the primary changes (Gelb *et al.*, 1973).

It did not appear likely that the decrease in MEF at 10% of VC was due to emphysematous changes with a concomitant loss of elastic recoil. Diffusing capacity was not decreased significantly in the exhaust exposed cats suggesting that there was no great loss of alveolar surface area. Pathological examination likewise failed to show any definitive signs of emphysema. Residual volume, which usually increases during the development of emphysema (Snider and Sherter, 1977), did show a moderate although not significant enlargement. Such an increase, however, may also occur with other conditions in which airway constriction inhibits emptying (Hogg *et al.*, 1968).

The lack of change in the frequency dependence of helium washout likewise indicated that the decrease in flow at low lung volume was probably not due to chronic obstructive lung disease. The basis for this test has evolved from the mechanical time constant concept of Otis *et al.* (1956). Basically, if resistance to flow in parallel small airways varies sufficiently, the distribution of gas to the lungs distal to these airways will vary with the rate of respiration. As respiratory rate increases, low resistance units receive greater portions of air resulting in less efficient washout. In chronic obstructive disease, resistance in parallel airways can vary widely due to partial or total blockage. This test is sufficiently sensitive to detect changes even in asymptomatic smokers (Huckauf and Jung, 1975). Thus, it was concluded that the only functional changes likely to have taken place in the lungs from the exhaust exposed cats was a small but uniform increase in small airway resistance.

The pathological findings were also limited in scope. The focal hypertrophy of the alveolar lining cells and interstitial thickening were not of a sufficient degree to result in an alteration of gas exchange as indicated by diffusing capacity. The presence of focal alveolitis characterized by the accumulation of pigmented macrophages was considered to be the most significant finding. The results indicated that following exposure to diesel exhaust, diesel particulate is found in the deep lung and can cause histopathological changes.

Despite the limited degree of both functional and pathological changes, it cannot be concluded that exposure to diesel exhaust emissions is not harmful to the lungs. Twenty eight days is a very short time period for the development

of pathological states such as emphysema and chronic obstructive pulmonary disease. Several years were required before emphysematous lesions could be detected in dogs exposed to automobile exhaust emissions at near ambient pollution levels (Hyde *et al.*, 1978). Even with direct instillation of a proteolytic enzyme into the lungs, the development of emphysema was minimal after 3 weeks but continued to progress for at least 6 mon. (Snider and Sherter, 1977). The findings reported here may therefore reflect merely the beginning of progressive pathological changes in the lung.

The present study is the first in a planned series of investigations designed to evaluate the potential health hazards of diesel exhaust inhalation. Further studies will include repeated measurements with exposure times of up to 2 years.

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REFERENCES

Amdur MO, Mead J. The respiratory response of guinea pigs to inhalation of ethylene oxide. Arch Environ Health 1956; 14:553-9

Bouhuys A. Breathing: Physiology Environment and Lung Disease. eds New York: Grune and Stratton 1974 pp.174

Creasia DA, Poggenburg JK, Nettesheim P Jr. Elution of benzo (a) pyrene from carbon particles in the respiratory tract of mice. J Toxicol Environ Health 1976; 1:967-75

DaSilva AMT, Hamosh P. Effects of smoking a single cigarette on a small airways. J Appl Physiol 1973; 34:361-365

Federal Register. vo. 31, No. 61, Paragraph 85, 72. March 30, 1966

Gelb AF, Gold WM, Wright RR, Bruch HR, Nadel JA. Physiologic diagnosis of subclinical emphysema. Am Rev Resp Dis 1973; 107:50-63

Hinners RG, Burkart JK, Punte CL. Animal inhalation exposure chamber. Arch Environ Health 1968; 14:533-9

Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructure lung desease. N Engl J Med 1968; 278:1355-61

Huckauf H, Jung J. Frequency dependent changes in gas distribution revealing early mechanical alterations in human lungs. Pneumologie 1975; 152:291-8

Hyde HD, Orthoefer J, Dungworth D, Tyler W, Carter R, Lum H. Morphometric and morphological evaluation of pulmonary lesions in beagle dogs chronically exposed to high ambient levels of air pollutants. Lab. Inves. 1978; 38:455-69

Karasek FW, Smythe RJ, Laub RJ. A gas chromatographic-mass spectometric study of organic compounds absorbed on particulate matter from diesel exhaust. J Chromatograph 1974; 101:125-36

McFadden FRR, Kiker R, Homes B, Degroot WJ. Small airway disease: An assessment of tests of peripheral airway function. Amer J Med 1974; 57:171-82

Moorman WJ, Lewis TR, Wagner WD. Maximum expiratory flow-volume studies on monkeys exposed to coal dust. J Appl Physiol 1975; 39:444-8

Ogilvie M, Foster RE, Blakemore WS, Morton JW. A Standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide, J. of Clinical Invest. 1957; 36:1-17

Otis AB, McKerrow CB, Bartlett RA, Mead J, McIlray J, Silverstone MB, Radford EP Jr. Mechanical factors in distribution of pulmonary ventilation. J Appl Physiol 1956; 8:427-43

Snider GL, Sherter CB. A one-year study of the evolution of elastase-induced emphysema in hamsters. J Appl Physiol 1977; 43:721-9

Stokinger HE. Toxicology of Diesel Emissions. Proceedings of the Symposium on Use of Diesel-Powered Equipment in Underground Mining. Pittsburgh, PA, Jan 30-31st 1973; pp. 146-58

Task Group On lung dynamics: Deposition and retention models for internal dosimetry of the human respiratory tract. Health Phys 1966; 12:173-207

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